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GENETIC ENGINEERING OF COTTON TO INCREASE FIBER STRENGTH, WATER ABSORPTION AND DYE BINDING

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GENETIC ENGINEERING OF COTTON TO INCREASE FIBER STRENGTH, WATER ABSORPTION AND DYE BINDING

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Cross Reference to Related Application

This patent application benefits of the filing date of provisional application serial No. 60/0174,997 filed February 17, 1998, entitled Genetic Engineering of Cotton to Increase Fiber Strength, Water Absorption and Dye Binding, attorney docket No. 922.6641PROV. That earlier application is incorporated herein in its entirety.

Field of the Invention

The invention relates to genetic engineering of cotton to increase the cotton's fiber strength, water absorption and dye binding.

Description of the Related Art

The description of the related art is listed under References towards the end of this document. All references cited herein are incorporated by reference.

Background of the Invention

About 20 million metric tons of cotton fiber is produced annually worldwide with the U.S. producing about 20% of this. Approximately 16 million acres of cotton are planted in the U.S. representing one-seventh of the world acreage. The United States generates one fifth of the worldwide cotton fiber production, valued at about four billion dollars annually. Cotton is the premier natural fiber and provides excellent wearability and aesthetics. Although consumers prefer cotton, man-made fibers have captured a major share of the textile market while the market share of cotton is decreasing. Improvements in cotton fiber strength, the chemical reactivity for dye binding, water absorption and thermal properties are desirable for textile and other industrial applications. In the past, cotton fiber quality has been improved by classical plant breeding; however, this approach is seriously limited by species incompatibility and available traits. An alternative approach is to introduce foreign genes to confer desired traits into cotton via genetic engineering. Recently, John and Keller (1996) have reported expression of polyhydroxy butyrate polyester in cotton fiber, which has similar physical and chemical properties as polypropylene. This is the first report of a foreign gene expression in cotton fiber.

Cotton fiber or seed hair is a terminally differentiated single epidermal cell made up of primary and secondary cell walls, consisting of primarily cellulose (90%) and other compounds like hemicellulose, pectins and proteins. During the early stages of fiber development, the fiber cell elongates up to 3 cm over a period of 20 days post anthesis (DPA). The primary wall is about 100-200 molecules in thickness and consists of 30% cellulose and other polysaccharides, waxes and proteins (John and Keller, 1996). The secondary wall is made up of cellulose that is deposited during the third developmental stage, 16-45 DPA. Maturation of the fiber occurs 45-50 DPA, resulting in changes in mineral content and protein levels. The chemical composition and microstructure of primary and secondary walls influence properties like chemical reactivity, thermal characteristics, water absorption and fiber strength (John, 1995b), which are important for the manufacturing of textile products. Therefore, it is highly desirable to synthesize a biopolymer within the fiber lumen without altering fiber wall integrity; this should result in sheltering the biopolymer within the cellulose walls (John and Keller, 1996). We propose here to introduce a PBP from a synthetic gene into cotton that could increase fiber strength, alter thermal and water absorption qualities as well as enhance dye binding capacity of cotton fiber.

Summary of the Invention

The United States generates one-fifth of the world's cotton fiber valued at about four billion dollars annually. Although consumers prefer cotton, man-made fibers are capturing a major share of the textile market while the market share of cotton is shrinking. In order for the market share to increase, cotton fiber quality must be improved. In the past, cotton fiber quality has been improved by classical plant breeding; however, this approach is limited by species incompatibility and available traits. An alternative approach is to introduce foreign genes to confer desired traits into cotton via genetic engineering. Protein-based polymers (PBPs) are available in nature as materials with extraordinary mechanical properties, such as spider webs composed of silk threads tougher than steel and elastin, a rubber like elastic fiber found in human arteries, that typically survives for more than 70 years, undergoing repeated cycles of stretching and relaxation. The PBP made from synthetic genes, containing the sequence Val-Pro-Gly-Val-Gly, typically found in elastin, exhibits elastic moduli that can range from 10^6-10^9 dynes/cm^2 and temperature transition properties that enable water absorption 10 times its own weight. Therefore, introducing this PBP into cotton fiber will increase the fiber strength, water absorption, thermal characteristics and dye binding. In this project, we attempt to genetically engineer cotton fiber with a PBP gene, GVGVP, the sequence derived from human elastin.

Description of the Invention

PBPs containing multiple repeats of the pentamer sequence (Val^1-Pro^2-Gly^3-Val^4-Gly^5)$_n$ exhibit remarkable elastic properties qualifying their use as bioelastic materials (Urry, 1995). Elastic and plastic PBPs offer a range of materials similar to that of oil-based polymers, such as hydrogels, elastomers and plastics. PBPs of varied design and composition can be prepared and made biodegradable with chemical clocks to program their half lives (Urry, 1995). Additionally, PBPs exhibit remarkable biocompatibility, thereby enabling their use in a whole range of medical applications including the prevention of post-surgical adhesions, tissue reconstruction and programmed drug delivery (Urry et al., 1993). For instance, the polymer poly (GVGVP) has been successfully used to prevent adhesions in the rat contaminated peritoneal model following abdominal injury (Urry et al., 1993). The non-medical application of these materials include biodegradable plastics, transducers, molecular machines, superabsorbant agents, and controlled release of agricultural crop enhancement agents, such as pesticides, growth factors and fertilizers (Daniell, 1995). Biodegradable plastics made from PBPs may not only break down in the environment but can become a useful part of the environment. Since saline solution can breakdown PBPs, the plastic PBP products can be disposed in oceans and gulfs; as they degrade, the plastics can provide proteins for oceanic animals, thus entering the food chain and benefiting the marine ecosystem.

Figure 1: PBP expression in E.coli

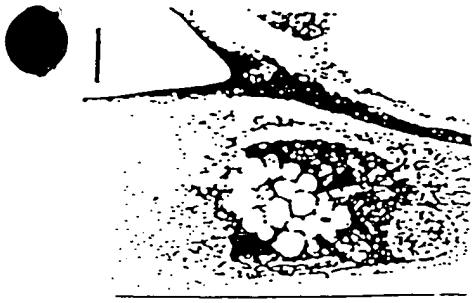

Figure 2: PBP expression in a plant cell

We have expressed the gene encoding poly $(GVGVP)_{121}$ in different systems including bacteria (Daniell, 1995; Guda et al., 1995; Daniell et al., 1997; Urry et al., 1995), fungi (Herzog et al., 1997) and plants (Zhang et al., 1995, 1996; Daniell, 1995; Daniell and Guda, 1997). Following expression of a small, 100 amino acid polypeptide $(GVGVP)_{20}$ in *E. coli* (McPherson *et al., 1992*), larger versions of the same polypeptide (GVGVP) containing 121 repeats (605 amino acids) or 251 repeats (1255 amino acids) were hyperexpressed in *E. coli* (Guda et al., 1995; Brixey et al., 1997). Bacterial cells showed polymer inclusion bodies occupying up to 90% of their cell volume under optimal conditions (See Figure 1). Production of polymers by fermentation, however, is not cost effective when compared with petroleum based polymers. Therefore, we have recently expressed the GVGVP 120mer in tobacco. Even though lower levels of expression were observed in cultured tobacco cells (Zhang et al., 1995) and some transgenic plants in the F0 generation (probably due to the position effect and heterozygous nature, Zhang et al., 1996), higher levels of polymer expression were observed in transgenic plants after self-crossing in the F1 generation; inclusion bodies have been observed in tobacco cells (see Figure 2), which is a good indication of a very high level of PBP expression (Daniell, 1995; Daniell, and Guda, 1997). The transgenic plants expressing this PBP grew, flowered and produced seeds normally (Zhang et al., 1996). Physiological and ultrastructural studies reveal that transgenic tobacco plants expressing the PBP are similar to control untransformed plants.

Rationale and Significance

PBPs are available in nature as materials with extraordinary mechanical properties, such as spider webs composed of silk threads tougher than steel, elastin fibers in the mammalian cardiovasculature which can last almost a century without loss of function and the adhesive produced by a mussel's foot which consistently adheres under extreme conditions in salt water. Elastin, a rubber like elastic fiber found in human arteries (especially in the aortic arch) typically survives for more than 70 years, undergoing repeated cycles of stretching and relaxation. The pentamer peptide sequence Val-Pro-Gly-Val-Gly is typical of all sequenced mammalian elastin proteins and in bovine elastin, this sequence is repeated eleven times without a single substitution. It has been shown that this elastic and plastic PBP exhibits elastic moduli that can range from 10^6-10^9 dynes/cm^2. Therefore, if this protein is introduced into the cotton fiber, it will confer an extraordinary level of elasticity and fiber strength, just like elastin.

These PBPs also exhibit temperature transition properties; parts of the polymer are hydrophobic and others are hydrophilic and water molecules accordingly arrange themselves around these sections of the molecules in different configurations. The relative stability of these configurations changes with temperature and so does the preferred shape of the protein. For example, when genetically engineered cotton containing the PBP is worn by an individual, the polymer will experience an inverse temperature transition just below the normal temperature of skin. When liquid touches the inside surface of clothing, the polymer molecules will soak it up, but they would remain in the folded state. The polymer chain's propensity to unfold at lower temperatures will spontaneously wick fluid away from the warm body and toward the cool outer surface of the clothing. Thus, this polymer can absorb 10 times its own weight in water (Urry, 1995). Moisture and water uptake by textile fibers are very important in regard to dyeing and finishing as well as for comfort and wearability. Water acts as a vehicle in the pores of the cellulose fiber for transport of dyes and other chemicals. Water absorption is directly correlated with fiber dyeability; reactive dyes form non-covalent bonds with functional groups along the

poly[redacted]backbone (Rivlin, 1992). Dye binding capacity will be [redacted]ced by increased protein content of the fiber; expression of the PBP in cotton fiber will significantly increase the fiber protein content. Based on all these observations, it is evident that introducing PBPs into cotton fiber will increase the fiber strength, water absorption, thermal characteristics and chemical reactivity.

In this context it should be pointed out that Agracetus, Inc. recently has introduced the polyhydroxybutyrate polymer biosynthetic genes into cotton for polyester expression in fiber (John and Keller, 1996). However, their genetic engineering approach, in addition to introducing a group of genes for the entire pathway, is limited by low levels of required intermediates (such as acetyl CoA) in the cytosol (Nawrath et al. 1995) resulting in very low levels of expression (0.3% fiber weight, John and Keller, 1996). Furthermore, properties of this polyester can not be modified to suitably alter fiber quality because the polyester is an end product of a bacterial pathway. In contrast, we attempt here to express a protein polymer and not a polyester. PBPs used in our study are expressed from a single synthetic gene that can easily be altered to increase the fiber strength, water absorption, thermal properties, elasticity and dye binding capacity of cotton fiber by changing the amino acid composition. We attempt to accomplish this using the gene $(GVGVP)_{121}$; this gene has been expressed at high levels in bacteria (Figure 1; Daniell et al., 1997) and plants (Figures 2; Daniell and Guda, 1997). Transgenic plants expressing this PBP grew, flowered and produced seeds normally (Zhang et al., 1996)

Objectives of this project: To Develop transgenic cotton plants with value-added traits:

a) Develop recombinant DNA transformation vectors for enhanced protein polymer expression in cotton fiber;
b) Obtain transgenic plants using the transformation vectors;
c) Assay transgenic expression using molecular and biochemical methods;
d) Assay fiber qualities of control and transgenic plants using physical and chemical testing, including fiber strength, elongation, water absorption and dyeability;
e) Analyze genetic composition of transgenic plants.

Discussion

Recombinant DNA vectors for PBP gene expression in cotton fiber

A nuclear vector for transient expression of the 120mer gene has been constructed. The plasmid pUC-GUS (obtained from Stratagene) was digested with XbaI and SstI to remove the 1.8 kb XbaI-SstI fragment containing the uidA gene, and the remaining 4.3 kb fragment was ligated with the 1.8 kb 120mer polymer fragment (obtained as XbaI-SstI fragment in pUC118) to produce plasmid pUC-XZ-120mer. The 120mer polymer gene in this construct is driven by the CaMV 35S promoter and flanked by the nos terminator. A nuclear vector for stable expression of the 120mer polymer protein also has been constructed. The uidA gene was removed from the plasmid pBI121 as a XbaI-SstI fragment and replaced by the 120mer polymer fragment (obtained as XbaI-SstI fragment in pUC118 plasmid) resulting in the construct pBI121-XZ-120mer (Figure 3). The 120mer polymer gene in this construct is driven by the CaMV 35S promoter and flanked by the nos terminator. This nuclear vector also contains a nptII gene driven by the nos promoter and flanked by the nos terminator to facilitate selection of transformed cells or tissues on kanamycin (see pBI121-XZ-120mer map shown below for more details).

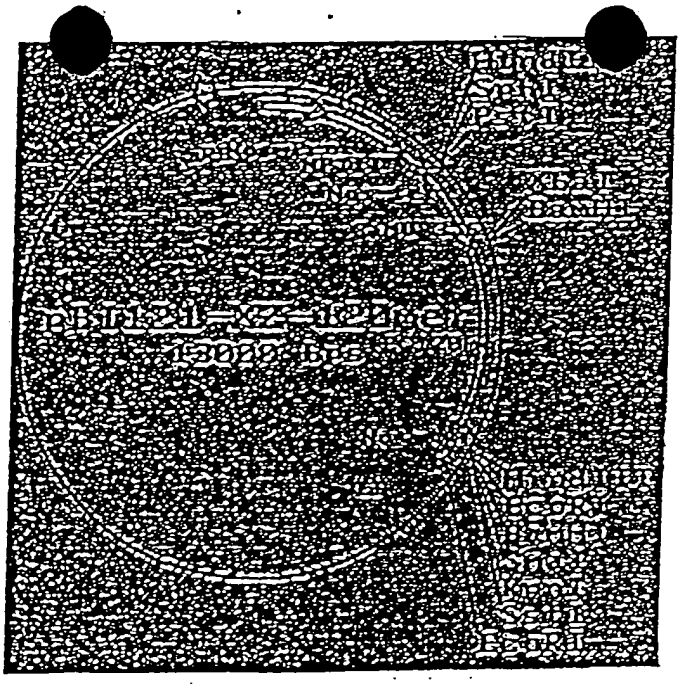

Figure 3: Plasmid map of pBI121-XZ-120mer

In stably transformed tobacco plants a 1.8 kbp EG-120mer polymer gene fragment was found to be integrated into the tobacco nuclear genome. A 1.8 kbp EG-120mer polymer gene transcript was observed in Northern blots. Gels stained with $CuCl_2$ show the presence of polymer and Western blots confirm the identity of the polymer protein (Zhang et al., 1995, 1996). Even though lower levels of expression were observed in cultured tobacco cells (Zhang et al., 1995) and some transgenic plants in the F0 generation (probably due to the position effect and heterozygous nature, Zhang et al., 1996), higher levels of polymer expression were observed in F1 transgenic plants after self-crossing. Inclusion bodies have been observed in tobacco cells (see Figure 2); this is a good indication of a high level of PBP expression (Daniell, 1995; Daniell and Guda, 1997). The transgenic plants expressing this PBP grew, flowered and produced seeds normally (Zhang et al., 1996). Physiological and ultrastructural studies reveal that transgenic tobacco plants expressing PBP are similar to control untransformed plants.

While the levels of PBP expression are sufficient in transgenic plants, we are attempting to further enhance the level of polymer production by modifying the codon composition. Therefore, the plant expression vector pBI-EV35S-130mer, with a plant nuclear preferred codon composition gene sequence, coding for the same polymer protein has been constructed in our lab and introduced into transgenic tobacco plants. Characterization of the tobacco transgenic plants expressing the 130mer polymer protein is in progress.

Identified fiber genes can be grouped into two types - genes which only express in fibers (fiber-specific genes) and those which express in other tissue types besides fibers. Fiber-specific genes, isolated from cDNA libraries, include a lipid transfer protein gene, the "fiber" gene E6 and Rac 13. However, only the promoter for the E6 gene, isolated from a genomic library, has been well characterized (John, 1995a). In order to avoid possible pleiotropic or epistatic effects of introduced genes, it is desirable to use promoters which will express foreign genes primarily in fiber cells. Therefore, in order to express PBP genes in cotton fibers, the 35S CaMV promoter in recombinant constructs (pBI121-XZ-120mer and pBI-EV35S-130mer) is replaced by the E-6 promoter (Figure 4); this will direct expression of foreign genes in a tissue specific and developmentally regulated manner in transgenic cotton plants (John and Crow, 1992). The E6 promoter has been used successfully to express PHB polymers in cotton fiber (John and Keller, 1996).

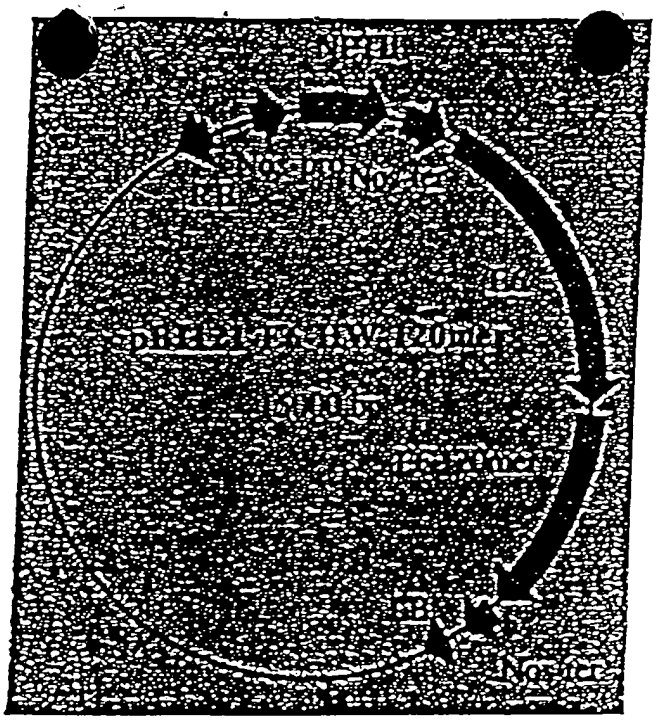

Figure 4: Plasmid map of pBI121-E6-HW-120mer

Cotton Transformation with PBP genes

Several methods for transformation of cotton in addition to the *Agrobacterium*-mediated transformation of hypocotyls have been described, including particle bombardment of embryogenic cultures and shoot apical meristems, followed by somatic embryogenesis or shoot formation from apical tissues, respectively. However, the *Agrobacterium*-mediated method followed by somatic embryogenesis (Trolinder and Goodin, 1987, 1988) remains the most reliable and manageable method in the university setting. In contrast, in the alternative method of particle bombardment (Daniell, 1997) of shoot apical meristems (McCabe and Martinell, 1993), thousands of bombardment events and repeated pruning of the resulting chimeric seedlings are required to produce uniform plants with transformed epidermal tissue or germ lines (John and Keller, 1996). The technical demands of the work are too great to be accomplished by the number of employees commonly supported in academic laboratories.

Alternatively, the *Agrobacterium*-mediated method is manageable in the university setting and has been used successfully to introduce 2,4-D resistance into cotton (Bayley et al, 1992). However, a disadvantage of this technique is that the subsequent regeneration is not cultivar-independent (Trolinder and Goodin, 1987, 1988). Consequently, desirable traits in the transformed plants must be subsequently crossed into current production varieties. After completion of recombinant DNA vector constructions, cotton transformation will be carried out in Dr. Haigler's laboratory (Texas Tech, Lubbock, TX). Fiber qualities of genetically engineered cotton will be analyzed at Auburn University and in collaboration with Dr. Rajasekaran, (USDA Southern Regional Laboratory, New Orleans, LA).

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